Pharmacokinetic profile of *Spectrum* reduced nicotine cigarettes

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ABSTRACT

Introduction: *Spectrum* research cigarettes have been developed with varying nicotine content for use in studies evaluating the effects of a regulatory policy reducing the permissible nicotine content in cigarettes. This study aimed to characterize the nicotine pharmacokinetic profile of *Spectrum* cigarettes.

Methods: 12 daily smokers attended 4 sessions and had blood nicotine, exhaled carbon monoxide (CO) and subjective effects measured before and after smoking either a single cigarette of their preferred brand or high (10.9 mg/cig), medium (3.2 mg/cig) or very low (0.2 mg/cig) nicotine content *Spectrum* research cigarettes, in a double-blind design with order counter-balanced.

Results: The boost in blood nicotine concentration was dose-dependent, with a boost of 0.3, 3.9 and 17.3 ng/ml for low, medium, and high nicotine content *Spectrum* cigarettes. The high dose *Spectrum* had a similar nicotine boost to the "preferred brand" cigarettes (19 ng/ml). Subjects took longer puffs on the low nicotine cigarettes, but smoked these cigarettes faster than other cigarette types. High nicotine *Spectrum* cigarettes reduced the urge to smoke more than other cigarette types.

Conclusions: This study shows that *Spectrum* research cigarettes produce blood nicotine absorption in a dose-dependent manner and therefore are appropriate for use in studies of nicotine reduction in cigarettes.

Implications: This is the first study to determine the pharmacokinetic profile of *Spectrum* reduced nicotine research cigarettes following an overnight abstinence. These data could provide evidence to regulatory agencies about the effects of reduced nicotine cigarettes when considering regulations on tobacco reduction.

INTRODUCTION

Cigarettes are addictive and nicotine is primarily responsible for this addiction[1]. To curb the negative health consequences of smoking cigarettes, a policy to gradually reduce the nicotine content of cigarettes has been proposed[2]. The proposal hypothesized that if the amount of nicotine in cigarettes was substantially reduced, it would help prevent the development of nicotine dependence in young people and aid in smoking cessation among addicted individuals attempting to quit. To achieve such an outcome, it was proposed that the nicotine content per cigarette should be reduced to 0.4 - 0.5 mg nicotine or lower[2]. It was reasoned that this level of nicotine would allow the taste and sensory stimulation of smoking to remain, but would result in cigarettes becoming minimally addictive.

The U.S. Food and Drug Administration recently proposed a nicotine regulatory strategy to reduce the addictiveness of cigarettes[3] and the FDA/NIH has funded a series of studies to evaluate the feasibility and effects of such strategies[4–7]. Most of these studies use *Spectrum* research cigarettes which are designed (through the use of genetically engineered tobacco plants) to contain specific amounts of nicotine for use in research. *Spectrum* research cigarettes are only available via the NIDA Drug Supply Program and are considered "Investigational Tobacco Products." Previous studies of reduced nicotine content *Spectrum* cigarettes have reported a reduction in cigarettes smoked among daily and non-daily smokers[8,9] as well as reductions in biomarkers of toxicant exposure and measures of dependence[4,7]. Critically, no published studies have examined the pharmacokinetic properties of *Spectrum* cigarettes. Knowledge of actual nicotine absorption will assist in understanding the results of randomized trials using *Spectrum* cigarettes.

This study set out to measure blood nicotine levels and accompanying behavioral and subjective measures after smoking a single *Spectrum* research cigarette in a laboratory setting in current smokers. Here we determine the pharmacokinetic profile of *Spectrum* reduced

nicotine cigarettes at three nicotine contents (very low = 0.2 mg/cig; medium = 3.2 mg/cig; high = 10.9 mg/cig nicotine) in comparison to the participants' usual brand[5,10,11]. We are unable to accurately determine the exact nicotine content of each participants' usual brand, however the average nicotine content of popular cigarette brands was reported as 10.2 mg/cig in 1998[12] and 13.9 mg/cig in 2005[13]. Finally, the participants answered a series of questionnaires related to the acute effects of smoking.

METHODS

Subjects

Subjects were 12 daily cigarette smokers (6 men/6 women). This sample size was chosen as it has been used to characterize the profile of blood nicotine levels following a single cigarette[14,15]. A sample size of 12 gives 80% power to detect a mean of paired differences of 15 ng/ml (SD=16) between the low and high dose Spectrum cigarettes, using a two-sided paired t-test. Participants were recruited if they smoked at least 10 cigarettes per day, which was verified by an expired carbon monoxide (CO) reading during a baseline session (described below). Subjects were excluded if they were anemic, reported having a respiratory illness exacerbated by smoking, were taking antidepressants, mood stabilizers or anxiolytic medication, or were pregnant. Individuals who reported using menthol or "roll your own" cigarettes were excluded. Participants were paid $10 for a baseline assessment and $250 for the completion of all four experimental sessions. When obtaining informed consent, participants were told the purpose of the study was to understand how smokers metabolize nicotine from reduced nicotine cigarettes and their reactions to such products. Participants gave informed consent and all procedures were approved by the Institutional Review Board of The Pennsylvania State University. One participant did not complete the own brand cigarette session.

Study Design

Baseline Session

Subjects completed an assessment battery including questions related to smoking history, demographics, and The Fagerström Test for Nicotine Dependence (FTND)[16]. Further, subjects had their expired CO level taken with a EC-50 Smokerlyzer (Bedfont Scientific, Williamsburg, VA) and were excluded if their CO level was <10 ppm. Hemoglobin levels were examined with a portable blood analyzer and participants with low levels were excluded (i.e., hemoglobin < 12.5 g/dL for females or < 13.0 g/dL for males). All testing was conducted in the Penn State Smoking Research Laboratory, a ventilated facility specially designed with negative air pressure to allow research participants to smoke indoors and have the air extracted and replaced quickly.

Experimental Sessions

Participants reported to the laboratory at 8 AM for each experimental session, conducted at least 2 days apart. Subjects were required to abstain from smoking for 12 hours before each session and instructed to not drink beverages that contain caffeine on the morning of their session. Upon arrival, subjects were required to provide an expired CO reading <10 ppm to verify overnight abstinence[17–19]. After confirming abstinence, an indwelling catheter was placed in the subject's arm by a trained nurse and a baseline blood sample (prior to smoking) was collected. The subjects were then instructed to smoke a single cigarette *ad libitum*, which consisted of either a *Spectrum* cigarette with very low (NRC 102 - 0.2 mg/cig nicotine), medium (NRC 400 - 3.2 mg/cig nicotine), or high nicotine content (NRC 600 – 10.9 mg/cig nicotine) or their preferred brand[11]. The *Spectrum* cigarettes were provided by the NIDA Drug Supply Program and normal brand cigarettes were purchased locally.

The order of cigarette presentation was randomized across participants using a Latin square design. The markings on the cigarette filter were covered with lab tape in order to blind the participant from the cigarette condition. The cigarettes were smoked through a SPA-M topography device (Sodim SAS, France) so that the number of puffs, puff duration, and duration of smoking data was recorded. The duration of smoking was obtained by instructing the participant to turn on the topography device when they started smoking and ending the recording when they finished their cigarette. Immediately after smoking was complete, repeated blood samples were taken at 2, 5, 10, 12, 25, 30, 60, 90, and 120 minutes to allow for the measurement of plasma nicotine levels. Plasma was stored at -20°C until analysis of nicotine levels by liquid chromatography-mass spectrometry[20] with a sensitivity limit of 0.0625 ng/mL and limit of quantitation of 0.2 ng/mL (signal/noise>=10). Finally, expired CO readings were taken at 12, 25, and 60 minutes after the experimental cigarette was smoked to measure CO absorption. Adherence to the protocol was insured by the researcher who observed the sessions through video monitoring of the smoking room.

To determine the psychological effects of smoking each cigarette, questionnaires were administered. Mood, craving, and withdrawal were assessed with The Questionnaire of Smoking Urges – Brief (QSU-B)[21–24], The Positive and Negative Affect Scale (PANAS)[25], and The Minnesota Nicotine Withdrawal Scale (MNWS)[26], respectively. The QSU-B and MNWS scores were calculated by adding each item-score into a total score. PANAS scores corresponded to the sum of all of the item-scores for the two scales: positive affect (items 1, 3, 5, 9, 10, 12, 14, 16, 17, and 19) and negative affect (items 2, 4, 6, 7, 8, 11, 13, 15, 18, and 20). These questionnaires were given before smoking (baseline) and 10, 30, and 60 minutes after cigarette completion.

To examine the subjects' reaction to the cigarettes, the Modified Cigarette Evaluation Questionnaire (mCEQ) was administered 10 minutes after smoking. With the mCEQ, smoking

satisfaction (Items 1, 2, and 12), psychological reward (Items 4 through 8), aversion (Items 9 and 10), enjoyment of respiratory tract sensations (Item 3), and craving reduction were examined (Item 11)[27].

Statistical Analysis

Linear mixed models were used to examine group differences for all dependent variables with LSD test for post hoc comparisons. Cigarette type and time were included in the analysis as repeated measures where appropriate. Topography dependent variables of interest include the time to smoke the cigarette (min), number of puffs taken, and average puff duration (s). For blood nicotine levels, dependent variables include the baseline plasma nicotine (ng/mL), nicotine boost (ng/mL) as calculated by the peak plasma nicotine level minus the baseline level, and the plasma area under the curve (AUC_{nic}). AUC_{nic} was calculated using the trapezoidal rule to 120 minutes. To correct for baseline nicotine levels, the nicotine half-life for each participant was calculated with the R package PKNCA. This half-life was then used to calculate the AUC of nicotine derived from the baseline and this was subtracted from the empirically measured AUC. Expired CO variables include baseline CO (ppm), CO boost (ppm) as defined by the peak expired CO minus the baseline CO, and the AUC_{CO} was calculated as defined for AUC_{nic}. Finally, behavioral data from the QSU-B, PANAS, and MNWS were calculated as the difference from post cigarette score minus the baseline (pre-cigarette) value. Raw scores on the mCEQ were analyzed as there were no baseline values. The significance threshold was set at alpha < 0.05. All analyses were performed in SPSS.

RESULTS

The subjects (mean age: 29 years; range: 18-55 years) smoked an average of 13.9 cigarettes per day (range: 10-20) for 9.8 years (range: 1.5-40). The average score on the Fagerström Test for Nicotine Dependence was 4.6 ± 1.6 (Range 2 – 7; Table 1).

Smoking Behaviors

Participants smoked low and medium nicotine content *Spectrum* cigarettes significantly faster than the high nicotine content *Spectrum* cigarette or their usual brand (Main effect of cigarette type: $F_{3,28}$ = 5.2; $p < 0.01$; Table 2). This difference in time to smoke the cigarette was not attributed to number of puffs, but the average puff duration was significantly longer on the low nicotine content *Spectrum* cigarettes compared to all other cigarette types (Main effect of cigarette type: $F_{3,28}$ = 4.9; $p < 0.01$; Table 2).

Pharmacokinetic Profile

There were no significant differences in baseline plasma nicotine levels, but there was a significant main effect of cigarette type on the magnitude of the nicotine boost ($F_{3,32}$ = 7.2; $p < 0.01$) and AUC_{nic} ($F_{3,32}$ = 25.7; $p < 0.001$; Fig 1, Table 2, and individual level data found in Supplementary Table 1). In both cases, the low and medium *Spectrum* cigarettes resulted in a lower plasma nicotine boost and AUC_{nic} compared to high or brand cigarettes (post hoc all $p < 0.05$). All four cigarette types produced a significant increase above the baseline plasma nicotine measure two minutes after smoking the cigarette (low *Spectrum* cigarette: t_{11} = -2.7, $p<0.05$; medium *Spectrum* cigarette: t_{11} = -4.0, $p<0.005$; high *Spectrum* cigarette: t_{11} = -2.3, $p<0.05$; brand cigarette: t_{10} = -4.2, $p<0.005$). In contrast to blood nicotine levels, the nicotine content of cigarette had no influence on CO parameters. There were no significant effects of cigarette type on baseline CO level, CO boost, or AUC_{CO}.

Modified Cigarette Evaluation Questionnaire

The participants reported no difference in enjoyment of the four cigarettes. The type of cigarette had no significant effect on satisfaction, reward, aversion, enjoyment of respiratory track sensations, or craving reduction subscales of the mCEQ (Table 2).

Minnesota Nicotine Withdrawal Scale

Smoking the high nicotine content *Spectrum* cigarette resulted in a greater decrease in withdrawal symptoms compared to all other cigarettes when corrected for baseline levels (reported as a change score; Fig 2A). We observed a significant main effect of cigarette type ($F_{3,120}$ = 4.07; p < 0.05). Smoking a high nicotine content *Spectrum* cigarette resulted in a greater reduction in withdrawal symptoms (mean ± SEM; -6.55 ± 1.02) compared to medium (-4.06 ± 1.03; post hoc p < 0.05) and very low (-4.41 ± 1.02; post hoc p < 0.05) nicotine content *Spectrum* cigarettes, or the participants' preferred brand (-3.16 ± 1.02; post hoc p < 0.05) independent of time after the cigarette.

Questionnaire of Smoking Urges – Brief

The decrease in urge to smoke was most prominent for the high nicotine content *Spectrum* cigarette 10 minutes after smoking. A main effect of cigarette type ($F_{3,120}$ = 4.06; p < 0.05) and time ($F_{2,120}$ = 9.02; p < 0.05) were observed. When participants smoked the high nicotine content *Spectrum* cigarette they had a greater reduction in urge to smoke (mean ± SEM: -18.97 ± 2.68) compared to the low or preferred brand cigarette (-10.27 ± 2.68 and -12.19 ± 2.68 respectively; Fig 2B, post hoc all p < 0.05). Additionally, the urge to smoke was significantly lower 10 minutes after smoking (-19.11 ± 2.53) compared to 30 (-12.77 ± 2.52; post hoc p < 0.05) or 60 min (-9.59 ± 2.52; post hoc all p < 0.05). No significant interactions between cigarette type and time were observed.

Positive and Negative Affect Scale

Smoking the high nicotine content *Spectrum* cigarette significantly decreased self-reported negative affect compared to all other cigarettes, including the participants' own brand (Fig 2C). Here a significant main effect of cigarette type ($F_{3,120}$ = 5.96, p<0.05) was observed, with high nicotine content *Spectrum* cigarettes showing a greater decrease in negative affect after smoking (Mean ± SEM: -4.94 ± 0.97) compared to very low nicotine content, medium nicotine content, or own brand (-2.97 ± 0.97, -1.58 ± 0.97, -1.86 ± 0.97 respectively; post hoc all p < 0.05). There were no significant main effects or interactions detected for positive affect (Fig 2D).

DISCUSSION

The primary goal of this study was to determine the pharmacokinetic profile *Spectrum* reduced nicotine research cigarettes following an overnight abstinence. Second, we report exploratory analyses of behavioral responses obtained during this experiment. Our results demonstrate that the boost in plasma nicotine concentration was reflective of the nicotine content of the cigarette, and AUC decreased as cigarette nicotine content decreased. Participants reported the experience of smoking the different cigarettes to be similar regardless of nicotine content, however the high nicotine content *Spectrum* cigarette was significantly more effective in relieving total withdrawal symptoms, urge to smoke, and negative affect. Surprisingly, no significant differences in symptoms of withdrawal were observed between the participants' preferred brand of cigarette compared to the low and medium *Spectrum* cigarettes. However, these subjective responses should be considered with caution due to our limited sample size.

Our data demonstrate important pharmacokinetic parameters of *Spectrum* research cigarettes. Specifically, we observed that high nicotine content *Spectrum* cigarettes resulted in a significantly greater nicotine boost and AUC compared to both the medium and low cigarettes. Additionally, there was a statistical trend (p = 0.08) for the medium cigarette to have a greater AUC compared to the low cigarette. We also found that the high dose (10.9 mg/cig) *Spectrum* research cigarettes provided a similar nicotine boost, behavioral puff topography, CO boost, and similar subjective effects to smokers' preferred brand cigarettes, suggesting that they provide an excellent "control" cigarette in double blind trials of the effects of reduced nicotine content in cigarettes.

Additional dose-response relationships have been detected on number of cigarettes smoked per day when low- and high-nicotine *Spectrum* cigarettes are available. For example, after 7 days of smoking *Spectrum* cigarettes, individuals given the low nicotine content cigarettes significantly reduced the number of experimental cigarettes smoked compared to those given the high nicotine research cigarettes[28]. Similar observations were observed in a 6-week randomized control trial, in which participants with access to low nicotine *Spectrum* cigarettes (< 2.4 mg/g) smoked fewer cigarettes per day compared to those with access to higher *Spectrum* cigarettes (15.8 mg/g)[29], suggesting that initial nicotine boost and subsequent bioavailability may mediate daily cigarette consumption[2]. However, our results for the medium *Spectrum* cigarettes are not in line with the observations from this clinical trial. Specifically, we found significant differences in nicotine boost and AUC between medium (3.2 mg/cig) and high (10.9 mg/cig) nicotine content *Spectrum* cigarettes. In contrast, Donny and colleagues reported no significant differences in number of cigarettes smoked between medium (5.2 mg/g) and high (15.8 mg/g) research cigarettes over a 6-week period, even when showing lower urinary total nicotine equivalents[29].

Previous studies have described differences in user's cigarette ratings based on the nicotine content of the cigarette[30,31]. Particularly, lower nicotine content *Spectrum* cigarettes have been reported to be less favorable on mCEQ subscales compared to higher nicotine content[28,32]. These studies measured subjective responses up to six weeks of regular use of *Spectrum* cigarettes[32] or after only four puffs on each cigarette[28], suggesting that repeated exposure does not alter this subjective responses. Our study's methodology differed from these published studies because our measurement was taken following an overnight abstinence. Further, these studies had a larger sample sizes. We did observe a trend (p = 0.079) toward increased reward in cigarettes with greater nicotine, suggesting that our results, on this subscale, are at least in a direction that is consistent with prior published findings.

Interestingly, our exploratory analyses revealed that high nicotine content *Spectrum* cigarettes showed a greater reduction in withdrawal, urge to smoke, and negative affect, compared to the other three cigarettes tested, including ones' preferred brand. This could be explained by the users' ability to discriminate between nicotine content as has been reported in previous studies[31,33–35]; at least within the *Spectrum* cigarettes. However, the user's own brand cigarette did not relieve withdrawal symptoms, urge to smoke, or negative affect when compared to medium and low nicotine content *Spectrum* cigarettes. This is inconsistent with prior work that has associated reduced nicotine content with relief of withdrawal symptoms[28], considering that users' own brand cigarettes contained similar amount of nicotine as the high *Spectrum* cigarettes. Future work using similar methodology and additional subjects would be required to confirm this finding.

The current study has some limitations. The study included only non-menthol moderate-to-heavy smokers, therefore our results are limited to this type of smokers. Further, the primary goal of this study was to examine the pharmacokinetic profile of *Spectrum* research cigarettes and the sample size was based on this goal. We reported changes in behavioral responses to

these cigarettes, however the statistical power for such analyses is limited and these results should be considered as exploratory. Finally, the present study was conducted following twelve hours of overnight abstinence. Although this is not a limitation for the pharmacokinetic analysis, for the behavioral results the participants were in a state of forced nicotine withdrawal, which could have moderated their subjective responses to the cigarettes. Nonetheless, our findings suggest that *Spectrum* research cigarettes produce blood nicotine absorption in a dose-dependent manner and are appropriate for use in studies of nicotine reduction in cigarettes.

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FIGURE LEGENDS

Fig 1. Blood nicotine profiles (Mean $\pm$ SEM) following smoking a single *Spectrum* cigarette with low (0.2 mg/cig nicotine), medium (3.2 mg/cig nicotine), or high nicotine content (10.9 mg/cig nicotine), or the participant's normal brand.

Fig 2. Smoking a high nicotine content *Spectrum* cigarette resulted in a greater alleviation of withdrawal symptoms, urge to smoke, and negative affect compared to other cigarette types. Data (mean $\pm$ SEM) represent (A) nicotine withdrawal scores on the MWS, (B) smoking urges on the QSU-B, and (C) negative and (D) positive affect on the PANAS for the three *Spectrum* cigarettes and the participants preferred brand at 10, 30 and 60 min after smoking the cigarette. All data are controlled for the baseline scores on each questionnaire. * $p < 0.05$ depicting a significant main effect of nicotine content. [#]$p < 0.05$ depicting a significant main effect of time.

Table 1. Participant demographics.

	Sample N (%)
Characteristics	
N	12
Male	6 (50)
Female	6 (50)
Race/Ethnicity	
White/Non-Hispanic	9 (75)
Asian/Non-Hispanic	2 (16)
More than 1 race/Hispanic	1 (8)
Education Level	
High School Graduate	3 (25)
Partial College (at least one year or specialized technical training)	3 (25)
College or University Graduate	5 (42)
Graduate or Professional Training	1 (8)
	Mean (SD)
Age (y)	29.0 (10.4)
Years of daily smoking	9.8 (11.3)
Cigarettes per day	13.9 (3.5)
FTND	4.6 (1.6)

Table 2. Smoking behavior and nicotine and carbon monoxide exposure from *Spectrum* cigarettes of differing nicotine content (N = 12 except where noted)

	Cigarette Type				ME of Type
	Very Low (0.2 mg/cig)	*Medium (3.2 mg/cig)*	*High (10.9 mg/cig)*	*UB*	*p-value*
Time to smoke cig (min)	2.7 (1.1)[#]	3.0 (0.6)[#]	3.7 (1.1)[#]	3.8 (1.0)[^]	P = 0.006 (UB, H > M, L)
No. of puffs	11.7 (6.0)[#]	12.0 (5.1)[#]	13.3 (3.8)[#]	14.9 (6.5)[^]	P = 0.098
Avg puff duration (s)	2.6 (0.8)[#]	2.2 (1.0)[#]	2.0 (0.6)[#]	2.1 (0.4)[^]	P = 0.007 (L > UB, H, M)
Baseline plasma nicotine (ng/mL)	1.6 (0.8)	1.7 (1.1)	2.4 (3.4)	1.8 (1.3)[#]	P= 0.633
Nicotine boost (ng/mL)	0.3 (3)	3.9 (3.3)	17.3 (25.3)	19.0 (13.2)[#]	P = 0.001 (UB, H > M, L)
AUC_{NIC} (ng/mL)	46.2 (32.7)	224.0 (142.1)	655.0 (470.4)	812.2 (394.4)[#]	P = 0.001 (UB, H > M, L)
Baseline CO (ppm)	6.25 (2.2)	5.4 (2.3)	5.5 (2.6)	5.4 (2.5)[#]	P = 0.463
CO boost (ppm)	5.0 (1.3)	4.9 (1.6)	4.9 (1.9)	6.1 (2.5)[#]	P = 0.148
AUC_{CO}	588 (140)	545 (178)	546 (234)	528 (234)[#]	P = 0.772
mCEQ Subscales					
Satisfaction	3.6 (1.7)	4.2 (1.9)	4.4 (1.8)	4.4 (1.7)[#]	P = 0.205
Reward	2.9 (1.6)	3.3 (1.7)	3.5 (1.8)	3.7 (1.5)[#]	P = 0.079
Aversion	1.5 (1.4)	1.6 (1.1)	1.8 (1.6)	2.0 (1.5)[#]	P = 0.525
Enjoyment of respiratory track sensations	4.0 (1.5)	3.9 (1.6)	4.3 (1.8)	4.5 (1.3)[#]	P = 0.780

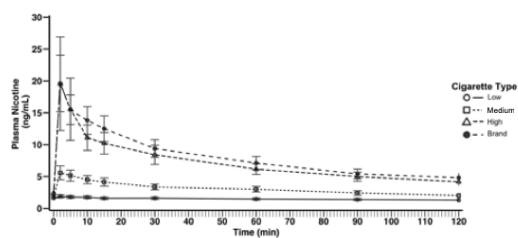| Craving reduction | 3.5 (1.7) | 4.6 (1.8) | 4.3 (2.0) | 4.5 (1.7)[#] | P = 0.469 |

Data are given as mean (SD).

UB, Usual Brand; AUC_{nic}, area under plasma nicotine concentration curve; CO, carbon monoxide;
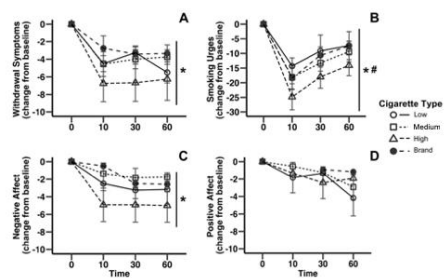
AUC_{CO}, area under blood carbon monoxide concentration curve.

n = 11, ^ n = 10

Figure 1

Figure 2